

Targeting the *tcdA* Gene: Is This Appropriate for Detection of A and/or B *Clostridium difficile* Toxin-Producing Strains?

In a recent article (3), Norén et al. reported an extensive evaluation of *illumigene Clostridium difficile* compared with the cell culture cytotoxin B assay (CTBA) and/or toxigenic culture (TC) of cytotoxin-producing *C. difficile* isolates. The *illumigene* assay utilizes loop-mediated isothermal DNA amplification (LAMP) technology to detect the pathogenicity locus (PaLoc) of toxigenic *Clostridium difficile*. The authors reported a sensitivity of 98% (49/50 isolates) and a specificity of 98% (218/222 isolates) for the *illumigene* assay, using combined CTBA plus TC as the gold standard.

While demonstrating good clinical performance, the authors raised the question of whether the toxin A fragment amplified by the *illumigene* assay is the optimal target for detecting toxin A-negative/toxin B-positive ($A^+ B^-$) strains. The *Clostridium difficile* PaLoc segment encodes both the toxin A gene (*tcdA*) and the toxin B gene (*tcdB*), has conserved border regions, and is found at the same site on the *C. difficile* genome for all toxigenic strains. Both the *tcdA* and the *tcdB* genes have similar structures consisting of three distinct fragments: a catalytic domain, a putative translocation domain, and a repetitive domain (4). The repetitive regions are prone to homologous recombination resulting in various deletions at the 3' ends of the toxin genes. For example, the well-characterized $A^- B^+$ strains (toxintypes VI and VII) and the most frequently occurring strains (toxintypes VIII and X) all have various deletions at the 3' end of the *tcdA* gene. However, the 5' portion of the *tcdA* gene remains intact for all of these strains. The rationale for the *illumigene* design is to target the 5' region of the *tcdA* gene that is present in all known $A^- B^+$ strains and seems to be more conserved than the *tcdB* gene (5).

Studies involving well-characterized *C. difficile* strains were conducted to confirm the primer design. The *illumigene* assay detected 4 $A^- B^+$ and 30 $A^+ B^+$ strains (2). Couturier and She (1) independently confirmed these findings. Again, the *illumigene* assay was able to detect toxin $A^+ B^+$ strains of toxintypes 0 (16 strains), III (6 strains), XII (1 strain), and IX/XXIII (1 strain). More importantly, it also amplified toxintypes VIII (3 strains) and X (1 strain), which are toxin $A^- B^+$ (40 non-*C. difficile* isolates were not detected, including 3 strains of *Clostridium sordellii*).

In summary, the *illumigene C. difficile* assay was designed to detect the genetically intact PaLoc region of toxigenic *C. difficile* regardless of expression phenotypes.

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Authors' Reply

In response to this issue's commentary by Ken Kozak, Chief Technical Officer at Meridian Bioscience, we wish to clarify some of our inherent caution when evaluating new diagnostic tests like *illumigene*. This promising loop-mediated isothermal DNA amplification (LAMP) assay was rapid and highly sensitive when performed on clinical specimens sent for *Clostridium difficile* analysis (1). We are well aware of the conserved nature of the target sequence of the toxin A gene (*tcdA*) (2) that allows for amplification of both truncated *tcdA* and phenotypically toxin A-negative *C. difficile* isolates. Our clinical experience to this date does not challenge this view.

From May 2010 to February 2011, we tested 674 clinical fecal specimens sent for *C. difficile* detection with both LAMP and toxigenic culture (TC) and in part with a cytotoxin B assay (CTBA) as described earlier (1). One side of the coin is the high sensitivity of a molecular test like LAMP, in which a case of *Clostridium difficile* infection (CDI) always has to be related to both clinical symptoms and the possibility of asymptomatic nosocomial carriage. Kozak and Elagin question the potential risk of LAMP missing a clinically relevant variant of *C. difficile*. The referred poster data of 4 toxin $A^- B^+$ detected strains is used in an attempt to reduce this concern. Up to this point, we have identified only 5 of 114 TC-positive specimens as LAMP negative by direct testing on feces (sensitivity, 96%). Out of these, 4 of 5 of the cultured *C. difficile* isolates tested positive in LAMP from the plate, indicating inactivation or possibly inferior sensitivity. A majority (3/4) of these specimens were from patients with a non-CDI type of diarrhea, and in two cases, the patients' specimens were very bloody, which is known to impact test results. One specimen is currently undergoing testing by toxintyping, since we failed to confirm the cell toxicity of a cultured isolate in CTBA.

In conclusion, LAMP provides the clinician with a very rapid and sensitive CDI diagnostic tool guiding therapy. There is, so far, no sign of false negatives relating to the specific PaLoc *tcdA* target design. Nevertheless, odd mutations can occur, and culturing of *C. difficile* isolates enables us not only to clarify phenotype but to test susceptibility and genotype the virulent strains as well.

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